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(57) Abstract

This invention provides a method of suppressing the malignant phenotype or inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein or fibroblast activating protein–α, thereby suppressing the malignant phenotype of the cancer. This invention provides a method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified dipeptidyl peptidase IV protein or fibroblast activating protein–α and a pharmaceutical acceptable carrier or diluent. This invention provides a method of inducing expression of dipeptidyl peptidase IV or fibroblast activating protein–α in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene or fibroblast activating protein–α and a pharmaceutical acceptable carrier or diluent.

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USE OF DIPEPTIDYL PEPTIDASE (DPP4) FOR SUPPRESSING THE MALIGNANT PHENOTYPE OF CANCER CELLS

The research leading to the present invention was supported, at least in part, by a grant from The National Cancer Institute, Core Grant No. CA 08748. Accordingly, the Government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to a method of suppressing the malignant phenotype of cancer cells, inducing apoptosis of cancer cells or treating a subject with cancer, comprising introducing into the cancer cell an amount of a nucleic acid encoding an ectopeptidase protein expressed on a cell membrane of a cancer cell, analogs, fragments, mutants thereof; or introduced into a subject with cancer; or a purified ectopeptidase protein or chimerics or fusion proteins. Ectopeptidases exemplified by this application include dipeptidyl peptidase IV protein, and fibroblast activating protein-α.

BACKGROUND OF THE INVENTION

Dipeptidyl peptidase IV is a cell surface peptidase that is expressed on epithelia, melanocytes, and T cells (1-3). It is a type II membrane glycoprotein that has multiple properties, including serine protease activity and ability to bind adenosine deaminase and extracellular matrix components (2-4). Because it binds to adenosine deaminase, DPPIV has also been called adenosine deaminase binding protein or adenosine deaminase complexing protein (4,5). DPPIV expression on T cells has been designated CD26 (3).

It has been recognized for several decades that expression of DPPIV can be extinguished or altered on cancer cells (6-8). Loss or alteration of membrane expression of DPPIV has been reported in prostate, colorectal, lung, and hepatocellular carcinomas, and melanomas (2,6-13). DPPIV expression during malignant transformation has been best characterized in melanocytic cells. DPPIV is expressed *in vitro* and *in vivo* by normal melanocytes but not by melanoma, the malignant counterpart (2,13). Previous studies have shown that expression of DPPIV is lost as melanocytes are transformed into

melanoma cells (2,13,14). Loss of DPPIV expression probably occurs at an early stage of melanoma progression as melanocytes transform into melanoma cells (2). Specifically, DPPIV is expressed by cutaneous melanocytes and common nevi, but is not detected *in vivo* or *in vitro* on cells from primary or metastatic melanomas. In an *in vitro* system that sequentially transformed melanocytes in defined steps, loss of DPPIV expression occurred concomitantly with the emergence of growth factor independence (14,13). However, despite these correlative observations both *in vivo* and *in vitro*, a role for DPPIV in regulating the malignant phenotype has not been shown.

BRIEF DESCRIPTION OF THE INVENTION

This invention provides a method of suppressing the malignant phenotype or inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein, thereby suppressing the malignant phenotype of the cancer.

This invention provides a method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified dipeptidyl peptidase IV protein, a purified fragment of a dipeptidyl peptidase IV protein, a purified mutant dipeptidyl peptidase IV, or a purified chimeric dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of inducing expression of dipeptidyl peptidase IV in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of increasing the level of dipeptidyl peptidase IV in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of treating a subject with cancer which comprises, administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

This is invention provides a method of suppressing the malignant phenotype or inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α , thereby suppressing the malignant phenotype of the cancer.

This invention provides a method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified fibroblast activating protein-α , a purified fragment of a fibroblast activating protein-α , a purified mutant fibroblast activating protein-α, or a purified chimeric fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of inducing expression of fibroblast activating protein-α in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the fibroblast activating protein-α gene and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of treating a subject with cancer which comprises, administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the fibroblast activating protein-α gene and a pharmaceutical acceptable carrier or diluent.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURES 1A-1C: Expression of DPPIV in transfected melanoma cells

Panel A. Immunoflourescence microscopy showing expression of DPPIV. MEL-22a cells were cultured in the presence or absence of doxycycline (2 μg/ml) for 48 hours and stained with S27 mAb against DPPIV. Groups include untransfected MEL-22a cells (panel 1) and control vector transfected cells (panel 2); cells transfected with wtDPPIV and grown in the absence of doxycycline (panel 3) or induced with doxycycline for 48 hours (panel 4); cells transfected with mutDPPIV grown in absence of doxycycline (panel 5) or induced with doxycycline for 48 hours (panel 6). Original magnification 400 x.

Panel B. Immunoprecipitation analysis of DPPIV expression in MEL-22a cells transfected with wt- or mutDPPIV. Cells were labeled with [^{35}S]-methionine for 18 hours, lysed with 1% NP-40, immunoprecipitated with mAb S27 against DPPIV, analyzed by 9% SDS-PAGE and visualized by autoradiography. Lanes 1 and 2 are untransfected MEL-22a and vector transfected MEL-22a controls respectively. Lanes 3 and 4 are two separate clones of transfected MEL-22a cells expressing either high (hi) or intermediate (med) levels of wtDPPIV when grown in the presence of doxycycline. Lane 5 is MEL-22a cells transfected with mutDPPIV grown in presence of doxycycline. Lane 6 shows wtDPPIV (hi) transfected MEL-22a clone grown in the absence of doxycycline. Arrow points to 110-120 kD band of DPPIV. Dox designates not induced (-) or induced (+) with doxycycline.

Panel C. Dipeptidyl peptidase IV activity in MEL-22a clones transfected with wtDPPIV, mutDPPIV, control vector and in cultured normal foreskin

melanocytes. Three different clones of MEL-22a cells transfected with wtDPPIV were analyzed expressing low (wtDPPIVlow), intermediate (wtDPPIVmed) and high (wtDPPIVhi) levels of enzyme activity. Open bars (-dox) show enzyme activities in absence of doxycycline. Enzyme activity after induction with doxycycline is depicted in hatched bars (+ dox). Results shown are mean values +/- 1 standard deviation of triplicates.

FIGURES 2A-2C: Effects of DPPIV expression on tumorigenicity.

Panel A. Expression of wtDPPIV was associated with inhibition of tumor growth of melanoma cells MEL-22a in nude mice. Six different sets of nude mice (BALB/C *nu/nu*, n=5-6 for each group) were challenged subcutaneously on day 0 with $3x10^6$ cells, either parental MEL-22a or transfected with wtDPPIV (with medium or high levels of expression induced by doxycycline as presented in Figure. 1), mutDPPIV, or control vector. Cells were induced (+dox) or not induced (-dox) with doxycycline for seven days prior to tumor challenge. Untransfected (MEL-22a) or vector transfected cells were used as controls. Tumor diameters were measured every 2-3 days. Results are presented as mean tumor diameter +/- 1 standard deviation.

Panel B. A repeat of the experiment shown in Panel A with 5-6 mice per group challenged with MEL-22a melanoma cells. The procedure was the same as that presented in Panel A.

Panel C. Tumorigenicity of SK-MEL-29 melanoma cells, including parental SK-MEL-29 cells and cells transfected with wtDPPIV, mutDPPIV or control vector. Tumor cells, $5x10^6$, were injected subcutaneously on day 0.

FIGURE 3: Anchorage independent growth in soft agar. MEL-22a cells described in panel A of Figure 2 were cultured in the absence (-) or presence (+) of doxycycline for 48 hours and 5000 viable cells plated in agar in triplicate as described in the Materials

and Methods. Results are mean number of colonies +/- 1 standard deviation at 14 days. Results are pooled from two separate experiments.

FIGURES 4A-4C: Phenotypic changes associated with DPPIV expression.

Panel A. Morphology of MEL-22a clones. Untransfected (panel 1) and control vector transfected cells (panel 2) showed short spindle shape and polygonal morphology, and grew in unorganized clusters. MutDPPIV transfected cells (panel 3) were morphologically similar to control cells. WtDPPIV transfected cells (panel 4) showed long spindle, bipolar morphology with more organized growth and sheet-like appearance. Original magnification 200x.

Panel B. Pigmentation of MEL-22a clones in cell pellets. Untransfected and control vector transfected MEL-22a cells were not melanotic (first and second pellets). Minimal pigmentation was observed in mutDPPIV transfected MEL-22a cells (third pellet). Expression of wtDPPIV led to brown pigmentation (fourth pellet). Dark brown pigmentation of normal foreskin melanocytes is shown in fifth pellet.

Panel C. Expression of human tyrosinase detected by Western blot. Lysates (1% NP-40) of human melanoma cell line G-MEL or MEL-22a cells, either parental or transfected with wtDPPIV (med and hi) or mutDPPIV or control vector, were separated on a 9% SDS/PAGE gel. After transfer to membrane, tyrosinase was detected as a broad ~75 kDa band using rabbit anti-PEP7H antibody against human tyrosinase. Left lane shows molecular mass markers.

FIGURE 5: Expression of DPPIV and FAPα. Expression of either wt- or mutDPPIV in MEL-22a cells rescued the expression of FAPα Immunofluorescence staining and flow cytometry analysis of DPPIV transfected cells were performed using mAb against DPPIV and FAPα. Cell clones are described in the legends of Figures 1 and 2. The vertical axis represents the relative cell number and the horizontal axis represents log

fluorescence intensity. Shaded curve represents control IgG1 antibody. Solid line represents DPPIV expression and dotted line represents FAPα expression.

DETAILED DESCRIPTION OF THE INVENTION

Dipeptidyl peptidase IV (DPPIV) is a cell surface peptidase expressed by normal melanocytes, epithelial cells, and other cells. Malignant cells, including melanomas and carcinomas, frequently lose or alter DPPIV cell surface expression. Loss of DPPIV expression occurs during melanoma progression at a stage where transformed melanocytes become independent of exogenous growth factors for survival. Tetracycline-inducible expression vectors were constructed to express DPPIV in human melanoma cells. Reexpressing DPPIV in melanoma cells at or below levels expressed by normal melanocytes induced a profound change in phenotype that was characteristic of normal melanocytes. DPPIV expression led to a loss of tumorigenicity, anchorage-independent growth, a reversal in a block in differentiation, and an acquired dependence on exogenous growth factors for cell survival. Suppression of tumorigenicity and reversal of a block in differentiation were dependent on serine protease activity, assessed using mutant DPPIV molecules containing serine to alanine substitutions. Surprisingly, dependence on exogenous growth factors was not dependent on serine protease activity. Reexpression of either wild type or mutant DPPIV rescued expression of a second putative cell surface serine peptidase, fibroblast activation protein-α, which can form a heterodimer with DPPIV. The results presented herein, demonstrate that downregulation of DPPIV is an important early event in the pathogenesis of melanoma.

This invention provides a method of suppressing the malignant phenotype of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding an ectopeptidase protein normally expressed on a cell membrane which is lost, decreased, or turned off in a cancer cell, analogs, fragments, mutants or peptides, thereof, thereby suppressing the malignant phenotype of the cancer.

This invention provides a method of inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding an

ectopeptidase protein expressed on a cell membrane which is lost, decreased, or turned off in a cancer cell, analogs, fragments, mutants thereof, thereby inducing apoptosis.

This invention provides a method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified ectopeptidase protein expressed on a cell membrane which is lost, decreased, or turned off in a cancer cell, analogs, chimerics, fragments, mutants, thereof, and a pharmaceutical acceptable carrier or diluent. In one embodiment the chimeric is a fusion protein. In another embodiment the fusion protein comprises an ectopeptidase and a Fc region of immunoglobulin, or a fragment thereof. In another embodiment the fusion protein comprises an ectopeptidase linked to a antibody, or fragment thereof.

Ectopeptidases which are expressed on cell surfaces of cells, include but are not limited to: neutral endopeptidase or aminopeptidase N. variant. In one embodiment one or more ectopeptidases are introduced into, or co-administered into a subject with, the cancer cell.

As defined herein "lost, decreased, turned off" means the complete absence; or decreased amount of the protein compared with the amount of the protein in a normal tissue; or the lack of expression in the normal location on the cell surface of the protein or functional activity in steady state mRNA encoding such protein.

As defined herein "suppressing the malignant phenotype" means anything which changes the traits of the cancer cell which contribute to its malignancy including but not limited to independence from growth factor requirements; anchorage independent growth; colony formation; or tumor formation in an animal.

A "nucleic acid" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double

stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5′ to 3′ direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA" is a DNA that has undergone a molecular biological manipulation.

As used herein, the term "cancer cell" means a "tumor cell" which refers to a neoplasm, *i.e.*, tissue that grows by cellular proliferation more rapidly than normal, *e.g.*, more rapidly than adjoining cells, or other cells in the tissue. Neoplastic cells continue to grow after growth stimuli cease. Generally, tumors represent or form a distinct mass of tissue. The present invention relates to both types of tumors, but is particularly valuable in the treatment of cancers. In one embodiment the cancer cells are selected from a group consisting of: melanoma; lymphoma; leukemia; and prostate, colorectal, pancreatic, breast, brain, or gastric carcinoma. Examples of tumors include but are not limited to: include sarcomas and carcinomas such as, but not limited to: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, germ tumor, non-small cell lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma,

hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. In a preferred embodiment the tumor is a melanoma.

This invention provides a method of suppressing the malignant phenotype of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein, thereby suppressing the malignant phenotype of the cancer.

This invention provides a method of suppressing the malignant phenotype of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent, thereby suppressing the malignant phenotype of the cancer in the subject.

This invention provides a method of inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein, thereby inducing apoptosis.

This invention provides a method of inducing apoptosis of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent, thereby inducing apoptosis.

This invention provides a method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified dipeptidyl peptidase IV protein, a purified fragment of a dipeptidyl peptidase IV protein, a purified mutant dipeptidyl peptidase IV, or a purified chimeric dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent.

In one embodiment the chimeric is a fusion protein. In another embodiment the fusion protein comprises dipeptidyl peptidase IV, a fragment of dipeptidyl peptidase IV, or a mutant dipeptidyl peptidase IV; and a Fc region of immunoglobulin, or a fragment thereof. In another embodiment the fusion protein comprises dipeptidyl peptidase IV, a fragment of dipeptidyl peptidase IV, or a mutant dipeptidyl peptidase IV linked to a antibody, or fragment thereof. Antibodies include but are not limited to: any antibody for targeting a tumor cell. For example, anti-GD3 for melanoma, prostate specific membrane antigen for prostate, siallylated TN for blood group, Lewis Y for epithelial cells, and anti-Her/Neu for breast.

The dipeptidyl peptidase IV mRNA, complete sequence is in Genbank Accession Number M74777 (see also Tanaka, et al. J. of Immunol. 149:481-486 (1992) which is incorporated by reference). A mutant dipeptidyl peptidase IV protein is a protein which lost normal function or gained different functional properties due to changes in the normal dipeptidyl peptidase IV sequence.

The term "polypeptide" or "protein" is used in its broadest sense to refer to a compound of two or more amino acids, amino acid analogs, or peptidomimetics. The amino acids may be linked by peptide bonds. In another embodiment, the amino acids may be linked by other the bonds, *e.g.*, ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If

the peptide chain is long, the peptide is commonly called a polypeptide or a protein. The polypeptide may have a methionine added.

The identity or location of one or more amino acid residues may be changed or modified to include variants such as, for example, deletions containing less than all of the residues specified for the protein, substitution wherein one or more residues specified are replaced by other residues and addition wherein one or more amino acid residues is added to a terminal or medial portion of the proteins. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

In another embodiment, the peptide or protein are from proteolytic digestion products of the protein. In another embodiment, the derivative of the protein has one or more chemical moieties attached thereto. In another embodiment the chemical moiety is a water soluble polymer. In another embodiment the chemical moiety is polyethylene glycol. In another embodiment the chemical moiety is mon-, di-, tri- or tetrapegylated. In another embodiment the chemical moiety is N-terminal monopegylated.

Attachment of polyethylene glycol (PEG) to compounds is particularly useful because PEG has very low toxicity in mammals (Carpenter et al., 1971). For example, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. A second advantage afforded by the conjugation of PEG is that of effectively reducing the immunogenicity and antigenicity of heterologous compounds. For example, a PEG adduct of a human protein might be useful for the treatment of disease in other mammalian species without the risk of triggering a severe immune response. The compound of the present invention may be delivered in a microencapsulation device so as to reduce or prevent an host immune response against the compound or against cells which may produce the

compound. The compound of the present invention may also be delivered microencapsulated in a membrane, such as a liposome.

Numerous activated forms of PEG suitable for direct reaction with proteins have been described. Useful PEG reagents for reaction with protein amino groups include active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate. PEG derivatives containing maleimido or haloacetyl groups are useful reagents for the modification of protein free sulfhydryl groups. Likewise, PEG reagents containing amino hydrazine or hydrazide groups are useful for reaction with aldehydes generated by periodate oxidation of carbohydrate groups in proteins.

In one embodiment, the amino acid residues of the protein described herein are preferred to be in the "L" isomeric form. In another embodiment, the residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of lectin activity is retained by the protein. NH_2 refers to the free amino group present at the amino terminus of a protein. COOH refers to the free carboxy group present at the carboxy terminus of a protein. Abbreviations used herein are in keeping with standard protein nomenclature, *J. Biol. Chem.*, **243**:3552-59 (1969).

Synthetic proteins, prepared using the well known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N^{α}-amino protected N^{α}-t-butyloxycarbonyl) amino acid resin with the standard deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield (1963, J. Am. Chem. Soc. 85:2149-2154), or the base-labile N^{α}-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han (1972, J. Org. Chem. 37:3403-3409). Both Fmoc and Boc N^{α}-amino protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art. In addition, the method

of the invention can be used with other N^{α}-protecting groups that are familiar to those skilled in this art. Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, Solid Phase Synthesis, Second Edition, Pierce Chemical Co., Rockford, IL; Fields and Noble, 1990, Int. J. Pept. Protein Res. 35:161-214, or using automated synthesizers, such as sold by ABS. Thus, protein s of the invention may comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (*e.g.*, β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc.) to convey special properties. Synthetic amino acids include ornithine for lysine, fluorophenylalanine for phenylalanine, and norleucine for leucine or isoleucine. Additionally, by assigning specific amino acids at specific coupling steps, α-helices, β turns, β sheets, γ-turns, and cyclic peptides can be generated.

The following amino acid analogs and peptidomimetics may be incorporated into a peptide to induce or favor specific secondary structures: LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a β-turn inducing dipeptide analog (Kemp et al., 1985, J. Org. Chem. 50:5834-5838); β-sheet inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:5081-5082); β-turn inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:5057-5060); ∝-helix inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:4935-4938); γ-turn inducing analogs (Kemp et al., 1989, J. Org. Chem. 54:109:115); and analogs provided by the following references: Nagai and Sato, 1985. Tetrahedron Lett. 26:647-650; DiMaio et al., 1989, J. Chem. Soc. Perkin Trans. p. 1687; also a Gly-Ala turn analog (Kahn et al., 1989, Tetrahedron Lett. 30:2317); amide bond isostere (Jones et al., 1988, Tetrahedron Lett. 29:3853-3856); tretrazol (Zabrocki et al., 1988, J. Am. Chem. Soc. 110:5875-5880); DTC (Samanen et al., 1990, Int. J. Protein Pep. Res. 35:501:509); and analogs taught in Olson et al., 1990, J. Am. Chem. Sci. 112:323-333 and Garvey et al., 1990, J. Org. Chem. 56:436. Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Patent No. 5,440,013, issued August 8, 1995 to Kahn.

The present invention further provides for modification or derivatization of the protein or peptide of the invention. Modifications of peptides are well known to one of ordinary skill, and include phosphorylation, carboxymethylation, and acylation. Modifications may be effected by chemical or enzymatic means. In another aspect, glycosylated or fatty acylated peptide derivatives may be prepared. Preparation of glycosylated or fatty acylated peptides is well known in the art.

Mutations can be made in a nucleic acid encoding the protein such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH_2 can be maintained.

Synthetic DNA sequences allow convenient construction of genes which will express analogs or "muteins". A general method for site-specific incorporation of unnatural amino acids into proteins is described in Christopher J. Noren, Spencer J. Anthony-Cahill, Michael C. Griffith, Peter G. Schultz, *Science*, 244:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

The protein or peptide may be derivatized by the attachment of one or more chemical moieties to the protein moiety. The chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular subcutaneous, intravenous, oral, nasal, pulmonary, topical or other routes of administration. Chemical modification of biologically active component or components may provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the component or components and decreasing immunogenicity. *See* U.S. Patent No. 4,179,337, Davis et al., issued December 18, 1979. For a review, *see* Abuchowski et al., in *Enzymes as Drugs* (J.S. Holcerberg and J. Roberts, eds. pp. 367-383 (1981)). A review article describing protein modification and fusion proteins is Francis, 1992, *Focus on Growth Factors* 3:4-10, Mediscript: Mountview Court, Friern Barnet Lane, London N20, OLD, UK.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol. copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose. dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene

oxide/ethylene oxide co- polymers, polyoxyethylated polyols and polyvinyl alcohol. Polyethylene glycol propionaldenhyde may have advantages in manufacturing due to its stability in water.

The antibody may be a monoclonal or polyclonal antibody. Further, the antibody may be labeled with a detectable marker that is either a radioactive, colorimetric, fluorescent, or a luminescent marker. The labeled antibody may be a polyclonal or monoclonal antibody. In one embodiment, the labeled antibody is a purified labeled antibody. Methods of labeling antibodies are well known in the art.

The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, the term "antibody" includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof.Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. Further the protein or antibody may include a detectable marker, wherein the marker is a radioactive, colorimetric, fluorescent, or a luminescent marker.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for the protein , or its derivatives, or analogs. Antibody fragments which contain the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

"Targeting molecule" as used herein shall mean a molecule which, when administered, localizes to desired location. In various embodiments, the targeting molecule can be a peptide or protein, antibody, lectin, carbohydrate, or steroid. In one embodiment, the targeting molecule is a peptide ligand of a receptor on the target cell. In a specific embodiment, the targeting molecule is a peptide comprising the well known RGD sequence, or variants thereof that bind RGD receptors on the surface of cells such as cancer cells, *e.g.*, human ova that have receptors that recognize the RGD sequence.

In another embodiment, the targeting molecule is an antibody. Preferably, the targeting molecule is a monoclonal antibody. In one embodiment, to facilitate crosslinking the antibody can be reduced to two heavy and light chain heterodimers, or the $F(ab')_2$ fragment can be reduced, and crosslinked to the DPP4 via the reduced sulfhydryl. Antibodies for use as targeting molecule are specific for cell surface antigen. In one embodiment, the antigen is a receptor. For example, an antibody specific for a receptor on cancer cells, such as melanoma cells, can be used. This invention further provides for the use of other targeting molecules, such as lectins, carbohydrates, proteins and steroids.

Antibodies can be labeled for detection *in vitro*, *e.g.*, with labels such as enzymes, fluorophores, chromophores, radioisotopes, dyes, colloidal gold, latex particles, and chemiluminescent agents. Alternatively, the antibodies can be labeled for detection *in vivo*, *e.g.*, with radioisotopes (preferably technetium or iodine); magnetic resonance shift reagents (such as gadolinium and manganese); or radio-opaque reagents. The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. The protein can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently

available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, ß-glucuronidase, ß-D-glucosidase, ß-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

This invention provides a method of inducing expression of dipeptidyl peptidase IV in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of increasing the level of dipeptidyl peptidase IV in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

In one embodiment the agent regulates the activation of a response element of the dipeptidyl peptidase IV gene. In another embodiment the response element is: interferon stimulated response elements (ISREs), butyrate response elements, NF-kB, c-myc, or c-myb. In another embodiment the agent is a cytokine or growth factor. In a preferred embodiment the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, interferon-γ or interleukin-13.

Other cytokines or growth factors include but are not limited to: IFN γ or α, IFN-β; interleukin (IL) 1, IL-2, IL-4, IL-6, IL-7, IL-12, tumor necrosis factor (TNF) α, TNF-β, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage CSF (GM-CSF); accessory molecules, including members of the integrin superfamily and members of the Ig superfamily such as, but not limited to, LFA-1, LFA-3, CD22, and B7-1, B7-2, and ICAM-1 T cell costimulatory molecules. Further, in another embodiment, this invention contemplates administering a demethylating agent. Examples of such include but are not limited to: 5-aza-2'-deoxycytidine, or 5-aza-2'-deoxycytidine in combination with a cytokine, such as interferon-γ.

This invention provides a method of treating a subject with cancer which comprises, administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

In one embodiment the agent regulates the activation of a response element of the dipeptidyl peptidase IV gene. In another embodiment the response element is: interferon stimulated response elements (ISREs), butyrate response elements, NF-kB, c-myc, or c-myb. In another embodiment the agent is a cytokine or growth factor. In another embodiment the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ. Further, in another embodiment, this invention contemplates administering a demethylating agent. Examples of such include but are not limited to: 5-aza-2'-deoxycytidine, or 5-aza-2'-deoxycytidine in combination with a cytokine, such as interferon-γ.

This invention provides a method of suppressing the malignant phenotype of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α , thereby suppressing the malignant phenotype of the cancer.

This invention provides a method of suppressing the malignant phenotype of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent, thereby suppressing the malignant phenotype of the cancer in the subject.

This invention provides a method of inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α, thereby inducing apoptosis.

This invention provides a method of inducing apoptosis of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent, thereby inducing apoptosis.

This invention provides a method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified fibroblast activating protein-α , a purified fragment of a fibroblast activating protein-α, a purified mutant fibroblast activating protein-α , or a purified chimeric fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent.

In one embodiment the chimeric is a fusion protein. In another embodiment the fusion protein comprises fibroblast activating protein, a fragment of fibroblast activating protein-α,or a mutant fibroblast activating protein-α; and a Fc region of immunoglobulin,

or a fragment thereof. In another embodiment the fusion protein comprises fibroblast activating protein-α a fragment of fibroblast activating protein-α, or a mutant fibroblast activating protein-α linked to a antibody, or fragment thereof.

This invention provides a method of inducing expression of fibroblast activating protein-α in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the fibroblast activating protein-α gene and a pharmaceutical acceptable carrier or diluent.

In one embodiment the agent regulates the activation of a response element of the fibroblast activating protein-α gene. In another embodiment the agent is a cytokine or growth factor. In another embodiment the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ.

This invention provides a method of treating a subject with cancer which comprises, administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the fibroblast activating protein-α gene and a pharmaceutical acceptable carrier or diluent.

In one embodiment the agent regulates the activation of a response element of the fibroblast activating protein-α gene. In another embodiment the agent is a cytokine or growth factor. In another embodiment the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ.

The fibroblast activating protein-α mRNA, complete sequence is in Genbank Accession Number U09278 (see also, Scanlan A., et al. P.N.A.S. 91: 5657-5662 (1994) which is incorporated by reference). Fibroblast activating protein-α is a cell surface type II integral membrane which contains a potential serine protease site.

This invention provides a method of increasing the level of dipeptidyl peptidase IV in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene or fibroblast activating protein-α (FAP-α) and a pharmaceutical acceptable carrier or diluent.

This invention provides a method of suppressing the malignant phenotype of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α in combination with an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent, thereby suppressing the malignant phenotype of the cancer in the subject.

A mammalian expression vector comprising a nucleic acid encoding dipeptidyl peptidase IV, a nucleic acid encoding a fragment of a dipeptidyl peptidase, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein under the control of an inducible promoter. In one embodiment the inducible promoter is a cytokine inducible promoter. In one embodiment the cytokine may be interleukin-4, tumor necrosis factor-α, or interferon-γ. In another embodiment, the expression vector may comprise a nucleic acid encoding a cytokine under the control of an inducible promoter. It is contemplated by this invention that the administration a dipeptidyl peptidase protein is co-administrated with an expression vector comprising a cytokine inducble promoter and a nucleic acid encoding a cytokine.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3′ direction) coding sequence. For purposes of defining the present invention, the promoter sequence is

bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and translated into the protein encoded by the coding sequence.

The nucleotide sequence coding for DPP4 protein, or pharmaceutically active fragment or analog thereof, can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Such elements are termed herein a "promoter." Thus, the nucleic acid encoding a DPP4 protein is operationally associated with a promoter in an expression vector of the invention and an inducible cytokine promoter. Both cDNA and genomic sequences can be cloned and expressed under control of such regulatory sequences. The necessary transcriptional and translational activators can be provided on a recombinant expression vector, or they may be supplied by the native gene encoding such activators..

Expression of DPP4 protein and/or cytokines may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host tumor cells in which expression is desired. Promoters which may be used to control immunomodulatory molecule gene expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), human cytomegalovirus (CMV)

promoter, the adenovirus major late promoter, the the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); and animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58), alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94), myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Once a particular recombinant DNA expression vector for use in the invention is prepared, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or

adenovirus, adeno-associated virus, retrovirus, and plasmid and cosmid DNA vectors, to name but a few. For example, a vector which comprises the above-described nucleic acid molecule. The promoter may be, or is identical to, a bacterial, yeast, insect or mammalian promoter. Further, the vector may be a plasmid, cosmid, yeast artificial chromosome (YAC), bacteriophage or eukaryotic viral DNA.

Other numerous vector backbones known in the art as useful for expressing protein may be employed. Such vectors include, but are not limited to: adenovirus, adeno-associated virus simian virus 40 (SV40), cytomegalovirus (CMV), mouse mammary tumor virus (MMTV), Moloney murine leukemia virus, DNA delivery sytems, i.e. liposomes, and expression plasmid delivery systems. Further, one class of vectors comprises DNA elements derived from viruses such as bovine papilloma virus, polyoma virus, baculovirus, retroviruses or Semliki Forest virus. Such vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art. In a preferred embodiment, the vector is a retrovirus, adenovirus, adeno-associated virus vector.

Vectors are introduced into the desired host cells by methods known in the art, *e.g.*, *ex vivo* viral vectors, particularly retroviral vectors, *in vivo* viral vectors, particularly defective viral vectors or adeno-associated virus vectors, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, U.S. Patent No. 5,580,859, which is incorporated by reference and Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990). Such vectors include an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. Defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can

infect other cells. Thus, a solid tumor can be specifically targeted. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector (Kaplitt et al., 1991, Molec. Cell. Neurosci. 2:320-330), an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al. (1992, J. Clin. Invest. 90:626-630), and a defective adeno-associated virus vector (Samulski et al., 1987, J. Virol. 61:3096-3101; Samulski et al., 1989, J. Virol. 63:3822-3828).

In another embodiment the gene can be introduced in a retroviral vector, *e.g.*, as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al., 1983, Cell 33:153; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., 1988, J. Virol. 62:1120; Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995, by Dougherty et al.; and Kuo et al., 1993, Blood 82:845. Retroviral vectors are especially attractive for transfecting solid tumors, since the cells of the tumor are replicating.

Alternatively, the vector can be introduced *in vitro* or *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner, et. al., 1987, Proc. Natl. Acad. Sci. U.S.A. 84:7413-7417; *see* Mackey, et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:8027-8031)). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, 1989, Science 337:387-388). The use of lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells, in this instance tumor cells, *e.g.*, via tumor-specific cell surface receptors, represents one area of benefit. Lipids may be chemically coupled to other molecules for the purpose of targeting (*see* Mackey, et. al., 1988, *supra*). Targeted peptides, *e.g.*, hormones or

neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

It is also possible to introduce the vector *ex vivo* or *in vivo* as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter (see, U.S. Patent No. 5,580,859, the contents of which are hereby incorporated by reference and *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

According to the invention, the component or components of a therapeutic composition of the invention may be introduced or administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermaly, subcutaneously, intraperitonealy, intraventricularly, or intracranialy. More preferably, the DPP4 protein, analog, chimeric, fragments, mutant, or peptide; or expression vector, may be introduced by injection into the tumor or into tissues surrounding the tumor.

Modes of delivery include but are not limited to: naked DNA, protein, peptide, or within a viral vector, or within a liposome. In one embodiment the viral vector is a retrovirus, adeno-associated virus, or adenovirus.

As can be readily appreciated by one of ordinary skill in the art, the compositions and methods of the present invention are particularly suited to treatment of any animal, particularly a mammal, more specifically human. But by no means limited to, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, etc., *i.e.*, for veterinary medical use.

As used herein, "pharmaceutical composition" could mean therapeutically effective amounts of protein products of the invention together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in SCF therapy. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of SCF. The choice of compositions will depend on the physical and chemical properties of the protein having SCF activity. For example, a product derived from a membrane-bound form of SCF may require a formulation containing detergent. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines) and SCF coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

Further, as used herein "pharmaceutically acceptable carrier" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of

non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

When administered, compounds are often cleared rapidly from mucosal surfaces or the circulation and may therefore elicit relatively short-lived pharmacological activity. Consequently, frequent administrations of relatively large doses of bioactive compounds may by required to sustain therapeutic efficacy. Compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound adducts less frequently or in lower doses than with the unmodified compound.

The preparation of therapeutic compositions which contain an active component is well understood in the art. Typically, such compositions are prepared as aersol of the protein s delivered to the nasopharynx or as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

A active component can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein or antibody

molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

A composition comprising "A" (where "A" is a single protein, DNA molecule, vector, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc.) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A+B species in the composition, most preferably at least about 99% by weight.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to reduce by at least about 15 percent, preferably by at least 50 percent, more preferably by at least 90 percent, and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the host. As is appreciated by those skilled in the art the amount of the compound may vary depending on its specific activity and suitable dosage amounts may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. In one embodiment the amount is in the range of 10 picograms per kg to 20 milligrams per kg. In another embodiment the amount is 10 picograms per kg to 2 milligrams per kg. In another embodiment the amount is 2-80 micrograms per kilogram. In another embodiment the amount is 5-20 micrograms per kg.

The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid).

In yet another embodiment, the therapeutic compound can be delivered in a controlled release system. For example, the protein may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Preferably, a controlled release device is introduced into a subject in proximity of the site of inappropriate immune activation or a tumor. Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

As can be readily appreciated by one of ordinary skill in the art, the methods and pharmaceutical compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, etc., *i.e.*, for veterinary medical use.

In the therapeutic methods and compositions of the invention, a therapeutically effective dosage of the active component is provided. A therapeutically effective dosage can be determined by the ordinary skilled medical worker based on patient characteristics (age, weight, sex, condition, complications, other diseases, etc.), as is well known in the art. Furthermore, as further routine studies are conducted, more specific information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, is able to ascertain proper dosing. Generally, for intravenous injection or infusion, dosage may be lower than for intraperitoneal, intramuscular, or other route of administration. The dosing schedule may vary, depending on the circulation half-life, and the formulation used. The compositions are administered in a manner compatible with the dosage formulation in the therapeutically effective amount. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1: Role for Dipeptidyl Peptidase IV in Suppressing the Malignant Phenotype of Melanocytic Cells

The results presented show that reexpression of DPPIV in human melanoma cells at levels comparable to those found in normal melanocytes produced profound phenotypic changes. These included abrogation of tumorigenicity, reemergence of requirements for exogenous growth factors to maintain cell survival, and removal of a block in cell differentiation. Using a point mutation in the active serine protease domain of DPPIV, it was observed that serine peptidase activity was required for most effects, but not for

cell survival. Reexpression of DPPIV rescued expression of a second putative surface peptidase, fibroblast activation protein-α (FAPα). The expression of fibroblast activation protein-α contributes to effects on cell survival in malignant cells.

MATERIALS AND METHODS

Wild type and mutant plasmid constructs

Tetracycline inducible expression vectors pUHG16-3 and pUHD172-1neo were kindly provided by Hermann Bujard (Heidelberg, Germany) (16). The plasmid pUHG16-3 has a CMV minimal promoter fused to a tetracycline operator (teto). Transcription is activated by the reverse tet repressor in the presence of tetracycline or doxycycline. pUHD172-1neo has a neomycin resistance gene and reverse tetracycline controlled transactivator. Full-length cDNA (2.3 kb) of human DPPIV was amplified by PCR and subcloned into the Xba I site of pUHG16-3 to create pDPPIV. The DNA sequence was identical to the human DPPIV sequences reported in GenBank (accession number M74777). The orientation of the insert was confirmed by DNA sequencing and restriction enzyme digests. Mutant DPPIV (pmuDPPIV, producing amino acid substitution of alanine for serine at codon 630, was constructed using the Quickchange Site directed mutagenesis kit (Stratagene, LaJolla,CA). The oligonucleotide primers used for site directed mutagenesis were 5' GCA ATT TGG GGC TGG GCA TAT GGA GGG TAC 3' and 5' GTA CCC TCC ATA TGC CCA GCC CCA AAT TGC 3'. Mutants were identified by DNA sequencing.

Establishment of human melanoma cells expressing DPPIV

Human melanoma cells and melanocytes were established and cultured as described (17-19). Human melanoma cell line MEL-22a, SK-MEL-28 and SK-MEL-29 were co-transfected with plasmid pUHD 172-1neo and empty vector pUHG16-3, pDPPIV or pmuDPPIV, and the plasmid. Lipofectamine reagent was used for transfections as described by the manufacturer (Gibco BRL Life Technologies, Grand Island, NY).

Immunofluorescence microscopy and flow cytometry

Cells were grown on chamber slides (Nunc, Inc. Naperville, IL), then stained with mAb S27 (4 μg/ml) against DPPIV or mAb TA99 against gp75^{TRP-1}, and incubated with FITC-conjugated rabbit anti-mouse IgG (DAKO, Carpenteria, CA). Stained cells were viewed with a Nikon Optiphot microscope. Flow cytometry was performed using FACScan (Becton Dickinson, San Jose, CA). Cells were stained with S27 mAb or F19 mAb (anti-FAPα) (20) and FITC-conjugated rabbit anti-mouse IgG.

Immunoprecipitation and Western blot analysis

For immunoprecipitation assays (21), cells were cultured in medium containing [^{35}S] methionine (NEN Dupont, Boston, MA) for 18 hours and cell lysates were precipitated with anti-DPPIV mAb S27. Western blot analysis was performed as described (21), using rabbit PEP7H antibody against human tyrosinase (Vincent Hearing, NIH, Bethesda, MD).

Dipeptidyl peptidase enzyme activity, in situ apoptotic cell detection by TUNEL assay, cell cycle analysis, anchorage-independent studies and growth curves.

DPPIV peptidase activity was measured by colorimetric assay (22). Briefly, cells expressing DPPIV in the presence (2 μg/ml for 2-4 days) or absence of doxycycline were suspended in lysis buffer containing 0.5% CHAPS. Untransfected and vector transfected cells were used as controls. Thirty μl of cell lysates were incubated with 10 μl of 10 mM substrate, Gly-Pro *p*-nitroanilide (Sigma, St. Louis, MO) at 37^{0}C for 30 minutes. Reactions were stopped with 250 μl of 10% trichloroacetic acid and the supernatants were mixed with 250 μl of 0.1% $NaNO_2$ and incubated at room temperature for 3 min followed by addition of 250 μl 0.5% ammonium sulfamate. At the end of 2 min incubation, 500 μl of 0.05% N-(1-naphthyl)ethylenediamine was added and *p*-nitroaniline release was measured at 540 nm. Peptidase activities were standardized based on the protein concentration and also on cell number. Protein concentrations were measured by the Bradford assay using the BioRad DC protein assay kit. Specific activities were expressed as picomoles per microgram protein per minute.

For apoptosis assays, cells were grown in plain RPMI medium without serum for 3, 8, or 15 days. TUNEL assay was performed using the APOPTAG kit (Oncor, Gaithersberg, MD). Percent apoptosis was calculated by FACScan (Becton-Dickinson, San Jose, CA). Evidence of apoptosis and percent of cells in each phase of the cell cycle were analysed by CellFIT and PC-LYSIS software (Becton-Dickinson San Jose, CA).

Growth curves were determined as described (18). Briefly, cells were plated at a density of $1x10^4$ cells per well in triplicate in 24 well plates. Every three days cultures were refed with fresh media. Cells were trypsinized daily for 10-12 days, stained with trypan blue, and viable cells counted. Time of doubling was determined from a least squares regression fit of cell number versus time during logarithmic growth phase.

Colony formation was performed in soft agar. Briefly, the top layer consisting of 5000 viable cells suspended in 0.3% agarose and RPMI 1640 with 20% fetal calf serum was overlaid on a 1% agarose layer in 35 mm culture plates. Fourteen days after seeding, colonies equal to or greater than 200 μm in diameter were counted under a light microscope. The data are presented as the mean of triplicate plates.

Tumor growth in vivo.

Nude mice (*nu/nu*, BALB/c) were injected subcutaneously with $3x10^6$ cells (either MEL-22a or SK-MEL-29) expressing mutant or wild type DPPIV and control cells. Five to six animals were used for each group. The tumors were measured every two to three days along the greatest diameter. All mouse experiments were performed under protocols approved by the Institutional Animal Care and Utilization Committee of Memorial Sloan-Kettering Cancer Center according to NIH animal care guidelines.

RESULTS

Establishment and characterization of melanoma cells expressing DPPIV: To define a possible functional role of DPPIV in melanocytic cells, a melanoma cells that expressed DPPIV in an inducible manner using tetracycline inducible vectors was established. Three human melanoma cell lines, MEL-22a, SK-MEL-28 and SK-MEL-29, derived

from metastatic lesions of different patients were selected for study. These melanoma cell lines are representative of more than 150 melanoma cell lines that were tested which do not express detectable DPPIV glycoprotein (2). In addition, the growth and differentiation of these three melanoma lines have been well characterized (17,18). These cell lines represent different stages of melanocyte/melanoma differentiation (17). They are either completely non-pigmented with a phenotype that corresponds to an immature stage of melanocyte differentiation (MEL-22a) , or minimally pigmented with a phenotype of an intermediate stage of melanocyte differentiation (SK-MEL-28 and SK-MEL-29) (17,18) (Table 1).

Table 1: **Characteristics of human melanoma cells expressing wild type and mutant DPPIV**

Cell Line	(# clones)	Morphology	Pigmentation
MEL-22a			
Parental	-	Short Spindle/Epithelioid	-
Vector	6	Short Spindle/Epithelioid	-
wtDPPIVhi	3	Long Spindle/Bipolar	+++
wtDPPIVmed	2	Long Spindle/Bipolar	++
wtDPPIVlow	2	Short Spindle	-
mut DPPIV	3	Short Spindle/Epithelioid	-
SK-MEL-28			
Parental	-	Long Spindle/Bipolar	-
Vector	4	Long Spindle/Bipolar	-
wtDPPIV	3	Polydendritic	+++
mutDPPIV	3	Long Spindle/Bipolar	-
SK-MEL-29			
Parental	-	Long Spindle/Bipolar	-
Vector	4	Long Spindle/Bipolar	-
wtDPPIV	2	Polydendritic	++
mutDPPIV	3	Long Spindle/Bipolar	-

clones, number of clones tested.

Morphology nomenclature is described (references 17,18).

Pigmentation: -, no detectable pigmentation observed in cell pellet (SK-MEL-28 and SK-MEL-29 had pigment only detectable by melanin assays; 18); + to + + +, pigment detected in cell pellet. Light pigmentation corresponds to +, and ++ and +++ to darker pigmentation (+++ to pellet of wtDPPIV in Figure. 4A) and ++++ equivalent to deeply pigmented pellet observed for melanocytes (Figure. 4B).

Melanoma cells were cotransfected with a neomycin resistant regulator plasmid and tetracycline inducible vector carrying: 1) the full length wild type (wt) DPPIV cDNA, 2) a mutant (mut) DPPIV having minimal serine protease activity (substitution of alanine for serine at codon 630 altering the catalytic domain), or 3) a control empty vector (16). Multiple clones that expressed each cDNA construct were isolated for each cell line (Tables 1 and 2). Transfected clones expressing empty vector and mutDPPIV were always relatively easy to derive, and at least three clones were established for each melanoma cell line. Transfected clones expressing wtDPPIV were more difficult to establish, suggesting that DPPIV expression affected cell survival or growth. Despite this difficulty, seven different clones expressing low, medium and high levels of DPPIV were selected from MEL-22a transfectants, and two clones each were isolated for SK-MEL-28 and SK-MEL-29.

Table 2: Range of DPPIV Enzyme Activity[1] (pM/min/μg protein (#clones tested)

	Parental		Vector		WtDPPIV		MutDPPIV	
Dox	-	+	-	+	-	+	-	+
Melanoma Line								
MEL-22a	10-30	10-30	10-30	10-30 (6)	20-60	60-310 (7)	20-40	20-60 (3)
SK-MEL-28	10-30	10-30	10-30	10-30 (4)	20-60	240-300 (3)	20-40	20-60 (2)
SK-MEL-29	10-20	10-20	10-20	10-20 (4)	20-40	180-220 (2)	20-60	20-60 (3)

[1] Parental or transfected melanoma cell lysates were tested for DPPIV activity as described in the Materials and Methods. Dox, doxycycline used for induction.

DPPIV expression was assessed by three methods: 1) immunofluorescence staining; 2) immunoprecipitation from metabolically labeled cells; and 3) enzymatic activity. Figure 1 shows DPPIV expression in representative clones of MEL-22a transfectants, with or without induction by doxycycline. DPPIV cell surface expression was substantially induced by doxycycline (Figure 1A). Melanoma cells transfected with wtDPPIV and mutDPPIV expressed the expected 110-120 kDa glycoprotein, showing that both the wild-type and mutant polypeptides were processed and expressed appropriately (Figure 1B). Furthermore, these results showed that mutDPPIV was stable and expressed at levels comparable to wtDPPI. A weak 110 kDa band was detected in parental cells and cells transfected with empty vector upon long exposure of autoradiographs (Figure. 1B), suggesting that melanoma cells can express very low levels of endogenous DPPIV.

Transfected clones and parental melanoma cells were assessed for DPPIV enzymatic activity (Table 2 and Figure. 1C). Parental and vector control transfected cells expressed $\leq$30 pM/min/μg protein of DPPIV activity, which represents very low endogenous DPPIV activity. In the absence of doxycycline, DPPIV activity in DPPIV transfected melanoma cells was $\leq$60 pM/min/μg protein. Peptidase activity of melanoma cells induced to express high level of wtDPPIV in the presence of doxycycline was 220-310 pM/min/μg protein (Table 2 and Figure. 1C). This level was comparable to that of melanocytes (300-350 pM/min/μg protein, range from three distinct assays) (Figure. 1C). Despite high expression of mutDPPIV protein (Figure. 1A and 1B), melanoma cells expressing mutDPPIV exhibited low levels of enzyme activity even in the presence of doxycycline ($\leq$60 pM/min/μg protein) (Table 2 and Figure. 1C). Transfected MEL-22a clones were isolated that expressed high (hi), medium (med) and low DPPIV activity for more detailed studies to compare phenotype and level of DPPIV expression (Figure. 1B and 1C).

In summary, levels of DPPIV expression were consistent between the three assays, showing that steady state level of protein expression corresponded to wtDPPIV enzymatic activity. There was low DPPIV enzyme activity in cells expressing

mutDPPIV, as expected. Results of DPPIV activity in transfected melanoma lines SK-MEL-28 and SK-MEL-29 showed similar levels as MEL-22a (Table 2). These results showed that: 1) the maximum level of DPPIV activity in transfected melanoma cells did not exceed levels expressed by cultured normal melanocytes (either normalized to protein concentration or when calculated on a per cell basis); 2) doxycycline induced DPPIV expression 5 fold or more; and 3) mutDPPIV expressed minimal or no enzyme activity.

Inhibition of tumorigenicity by expression of DPPIV: Tumorigenicity of melanoma cells expressing wtDPPIV or mutDPPIV was compared to control melanoma cells. Nude mice were injected subcutaneously with transfected and control MEL-22a or SK-MEL-29 melanoma cells (parental SK-MEL-28 melanoma cells do not form tumors in immune compromised mice). Parental and control vector melanoma cells formed progressive tumors in all mice. Figure 2A and 2B shows results from two different experiments for MEL-22a and Figure. 2C for SK-MEL-29. Tumorigenicity was essentially ablated in MEL-22a cells when DPPIV was induced to levels expressed by normal melanocytes (wtDPPIVhi). Mice showed no progression of tumors over 100 days (Figure. 2A and 2B), although viable tumor cells remained after 100 days. Similar results were observed with SK-MEL-29 expressing wtDPPIV (Figure. 2C). Tumor growth was also reduced in melanoma cells expressing medium level of DPPIV, although not as profoundly as for high levels of DPPIV (Figure. 2A and 2C). Transfected MEL-22a melanoma cells expressing low levels of DPPIV, either in the absence of induction of DPPIV (wtDPPIV hi-dox; Figure 2A) or constitutively (DPPIVlow +dox; Figure. 2B), showed slightly reduced tumor growth, perhaps either due to low levels of DPPIV activity or recruitment of FAPα which forms a heterodimer with DPPIV (as discussed below). Melanoma cells expressing high levels of mutDPPIV formed tumors at variable rates, with some mice showing inhibition of tumor growth (note error bars in Figure. 2A and 2C for mutDPPIV). These results were consistent with a requirement of DPPIV serine peptidase activity for complete inhibition of tumorigenicity. However inconsistent inhibition of tumorigenicity in melanoma cells expressing mutDPPIV suggested that some effects of DPPIV on *in vivo* tumor growth were possibly independent of DPPIV serine peptidase activity.

Inhibition of anchorage-independent growth by expression of DPPIV: Another characteristic of malignant cells is anchorage-independent growth. Expression of DPPIV led to a marked decrease in the ability of MEL-22a melanoma cells to grow in soft agar. DPPIV expression inhibited colony forming ability by ~75% in MEL-22a cells, with little inhibition of MEL-22a cells expressing mutDPPIV compared to parental and vector control cells (Figure. 3). Thus, serine peptidase activity was required to decrease anchorage-independent growth.

Phenotypic changes of melanoma cells expressing DPPIV: Marked morphological changes were observed in melanoma cells expressing wtDPPIV (Figure. 4 and Table 1). Parental MEL-22a melanoma cells and cells transfected with control vector or mutDPPIV were a disorganized array of epithelioid, polygonal, and short bipolar spindle shaped cells, and grew in piled colonies without any apparent organization (Figure 4A). Cells expressing medium or high levels of wtDPPIV were consistently long bipolar spindle shaped with organized growth behavior and sheet-like appearance, suggesting organization by cell to cell contact (Figure. 4A). SK-MEL-28 and SK-MEL-29 cells also changed morphology from the long spindle shape of parental, control vector and mutDPPIV transfected cells to a more mature polydendritic shape of cells expressing wtDPPIV (Table 1). The polydendritic shape is characteristic of well differentiated melanoma cells (17,18).

It was previously shown that MEL-22a cells have a block in differentiation associated with a non-pigmented, immature melanocytic phenotype (18). Five MEL-22a clones expressing medium and high levels of DPPIV were pigmented when grown to confluence (Figure 4B and Table 1). Three clones expressing mutDPPIV had no pigment. None of six clones with control vector nor parental cells were pigmented (Figure 4B and Table 1). Differentiation of melanocytic cells is characterized not only by appearance of pigmentation, but by expression of melanosome membrane glycoproteins involved in melanin metabolism. The best characterized glycoproteins are members of the tyrosinase family, including tyrosinase and tyrosinase-related proteins (TRP). Expression of wtDPPIV, but not mutDPPIV, correlated with a marked increased expression of human

tyrosinase (Figure. 4C). Expression of wtDPPIV (but not mutDPPIV) was also associated with *de novo* expression of the *brown* locus protein, $gp75^{TRP-1}$ (23) measured by indirect immunofluorescence staining. Expression of $gp75^{TRP-1}$ and upregulation of tyrosinase protein occur at a later stage in melanocyte differentiation, confirming that the tyrosinase low/$gp75^{TRP-1}$ negative MEL-22a had differentiated. Induction of pigmentation associated with expression of DPPIV was also observed in the two other melanoma cell lines, SK-MEL-28 and -29 (Table 1). These observations show that DPPIV expression is associated with a relief of the block in differentiation of melanoma cells.

Growth characteristics of melanoma cells expressing DPPIV : Expression of DPPIV did not affect growth of MEL-22a cells during logarithmic growth phase. The doubling time of cells expressing high wtDPPIV, mutDPPIV, and control vectors was 36-38 hours, and was exactly the same as parental MEL-22a in culture media containing serum (36 hours). However, melanoma cells expressing medium and high levels of wtDPPIV had a much longer lag period after plating before they entered logarithmic growth phase (4-5 days) compared to parental cells and melanoma cells expressing mutDPPIV and control vectors (1-2 days). Also, growth of wtDPPIV cells was inhibited when cells reached a confluent state, while parental melanoma cells and cells expressing mutDPPIV and control vector continued to grow and pile up after reaching confluency (Figure. 3A). Thus, the total cell number of wtDPPIV cells was decreased by 40% compared to control melanoma cells or mutDPPIV cells at days 10-14 after plating. This was due to the delay before entering logarithmic growth but also perhaps to inhibited growth upon reaching confluency. Thus, wtDPPIV expression did not affect log growth of MEL-22a cells, but did slow entry into rapid growth phase and appeared to induce some level of inhibition of growth at cell confluency. The difficulty in initiating growth might explain in part the difficulty in establishing transfected clones of melanoma cells expressing wtDPPIV.

Apoptosis and block of cell cycle in serum free conditions induced by DPPIV expression: Transformed cells are typically released from dependence on exogenous growth factors for survival during tumor progression (24). This characteristic applies to melanoma cells,

which have been shown to survive and grow in serum free culture medium without addition of exogenous growth factors while normal melanocytes die over 7-14 days when serum is withdrawn (25,26). It was previously shown that loss of DPPIV expression was associated with acquisition of growth factor independence during *in vitro* transformation of melanocytes (14,13). This observation demonstrated a correlation between DPPIV expression and a requirement for exogenous growth factors for survival, which was investigated by growing transfected and parental melanoma cells in serum free conditions.

WtDPPIV, mutDPPIV and control MEL-22a cells were serum starved with or without induction of DPPIV by doxycycline. Parental and vector control cells grew in serum free media with only low levels of detectable apoptotic cell death (~2-3% of cells showed DNA fragmentation by TUNEL assay over 15 days) (Table 3). A minor population of transfected melanoma cells not induced for wtDPPIV cells demonstrated cell death in serum free conditions (21% of cells at 15 days) (Table 3). However cells induced to express either wtDPPIV or mutDPPIV with doxycycline showed a marked, progressive loss of cell viability; the proportion of apoptotic cells was 15-18% at day 3, 45-53% at day 8, and 62-78% at day 15 (Table 3). Similar results were observed with SK-MEL-29 cells. Only 8% of control vector transfected cells were apoptotic at 8 days after serum withdrawal compared to 52% of cells expressing wtDPPIV.

Table 3: Apoptosis of MEL-22a human melanoma cells expressing DPPIV in serum free conditions

		% Apoptosis[1] Day		
Melanoma Line	**Dox**	**3**	**8**	**15**
MEL-22a parental	-	3.3	2	3.4
Vector	-	2.1	3	2.5
wtDPPIVhi	-	4	18	21
wtDPPIVhi	+	17.5	53	78
wtDPPIVmed	+	15	45	62
mutDPPIV	+	17	50	68

[1] Control (parental and vector transfected) MEL-22a cells or MEL-22a cells transfected with DPPIV constructs were assessed for apoptosis by TUNEL assay as described in the Materials and Methods. % apoptotic cells were measured by flow cytometry. Dox, induction with doxycycline.

The same set of cells was analyzed for cell cycle progression in serum free conditions. WtDPPIVhi expression induced a cell cycle arrest at G0/G1 phase, with 62-76% of the cells present in G0/G1 stage by day 8 compared to only 5-12% of control vector and parental cells in G0/G1 (range of % from duplicates of two experiments). Interestingly, cell cycle arrest at the G0/G1 stage was detected in 32% of cells expressing mutDPPIV, intermediate between wild type DPPIV and melanoma cells not expressing DPPIV. These results with mutDPPIV suggest that either some other function than serine protease activity of DPPIV is involved in apoptosis and in cell cycle arrest induced by serum withdrawal, or that DPPIV interacts with other molecules that mediate survival and cell cycle effects.

DPPIV rescues expression of FAPα: FAPα is a potential cell surface serine protease that is co-expressed with DPPIV by melanocytes. Loss of FAPα expression occurs concomitantly with loss of DPPIV expression during *in vitro* transformation of

melanocytes, and expression is also lost in primary and metastatic melanoma cell lines (14,15). DPPIV and FAPα can form heterodimers in addition to homodimers formed by DPPIV (15). Reexpression of either wt- or mutDPPIV by MEL-22a melanoma cells induced the cell surface expression of FAPα (Figure 5). The relative level of surface expression of FAPα corresponded to the level of DPPIV expression, irrespective of wild type or mutant forms. Thus, DPPIV rescued surface expression of FAPα.

DISCUSSION

Cell surface proteases are generally thought to participate in malignant transformation and cancer progression by facilitating invasion and metastasis. Inhibition of surface proteases can block tumor progression and aberrant expression can facilitate tumorigenesis (27). However, cell surface proteases may also have the opposite effect, suppressing the malignant phenotype (27). How cell surface proteases play a role in suppressing the malignant phenotype of human cancer without interacting with the extracellular matrix is not well characterized.

The results show that loss of DPPIV expression is directly implicated in suppressing the malignant phenotype of melanoma cells. A crucial question arising from these studies is how a cell surface peptidase might have such pleiotropic effects on the malignant phenotype of melanoma cells, reversing tumorigenicity, affecting the differentiation program, and changing decisions about survival without exogenous growth factors. DPPIV has several functions, including its serine peptidase activity, binding to extracellular matrix components, and complexing adenosine deaminase (3). Thus each of these particular functions, presumably handled by different domains of the protein, could contribute to suppression of the malignant phenotype. Serine->alanine mutation did not suppress tumorigenicity or anchorage independent growth nor reversed the block in differentiation, showing that serine peptidase activity is required for these phenotypic changes.

Reexpression of DPPIV led to apoptotic cell death upon serum withdrawal and cell cycle arrest. Unexpectedly apoptosis also was observed in cells expressing mutDPPIV, which

suggests that the rescue of FAPα as a heterodimer with DPPIV could explain at least part of these pro-apoptotic effects. Consistent with this view, a paralogue of FAPα is induced during tadpole tail resorption, which is essentially a massive program of cell death (28). This suggests that a pro-apoptotic role of FAPα might be conserved throughout vertebrate evolution. FAPα contains a potential serine protease site, but the function of FAPα protein, including peptidase activity, are not well characterized. It will be important to determine whether FAPα serine protease activity is important for pro-apoptotic effects induced by expression of DPPIV. It will also be important to identify downstream components that are involved in decisions about melanoma cell survival, and how DPPIV and FAPα participate in these decisions. Pathways for cell survival in melanocytes are not well understated. However, bcl-2 is probably a central mediator of resistance to apoptotic death in melanocytic cells (29,30), and one speculation is that DPPIV expression ultimately might intersect with bcl-2.

DPPIV expression may play a crucial role in checking cell growth of normal melanocytes. This is supported by the observations that loss of DPPIV correlates with growth factor independent proliferation of melanoma cells (14,13), as well as the experiments described above. One explanation is that DPPIV degrades growth factors required for survival of melanocytic cells. Since the experiments were done in strict serum free conditions, the most likely source of growth factors are autocrine factors secreted by melanoma cells. In prostate cancer, autocrine neuropeptides such as bombesin and endothelin-1 can stimulate the growth of prostate carcinoma cells, and these growth factors are inactivated by the cell surface metallopeptidase, neutral endopeptidase 24.11 (31). Chemokines are potential substrates for DPPIV, including RANTES, SDF-1α, SDF-1β, IP-10, MCP-1, -2, and -3, and GCP-2 (32-36). In addition, regulatory peptides, including glucagon-like peptide-1 and -2, neuropeptide Y and peptide YY are DPPIV substrates (37,38).

EXAMPLE 2: USE OF CYTOKINES TO INDUCE EXPRESSION OF DPP-IV IN MELANOMA CELLS

Despite the low to absent expression of DPP-IV activity in cultured melanoma cells, southern blot analysis revealed that the gene coding for DPP-IV is intact. In addition, analysis of steady state mRNA using northern blots demonstrated that the DPP-IV message is present in melanoma cells, but in very low quantities. The above data suggest that DPP-IV is transcribed at very low levels due to lack of activation signal(s) or through the effects of a transcriptional repressor. One possible means of inducing expression of DPP-IV and reversing the malignant phenotype would be to identify exogenous agents (cytokines, growth factors) capable of activating transcription of the DPP-IV gene.

The promoter region of the human DPP-IV gene has been cloned and sequenced (Bohm et al., 1995, Biochem. J. 311, 835-843.). Careful inspection of this sequence reveals the presence of specific binding sites for several known transcription factors. These include an NF-κB site, several potential interferon stimulated response elements (ISREs), butyrate response elements, as well as c-myc and c-myb recognition sequences. The various transcription factor binding sites all represent potential mechanisms for upregulating the expression of DPP-IV in melanoma cells.

Experiments were conducted to examine the effects of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and interleukin-4 (IL-4) on melanoma cells with respect to DPP-IV expression and growth regulation/apoptosis (Fujiwara et al., 1994, J. Clin. Endo. Met. 7, 1007-1011.; Riemann et al., 1995, Clin. Exp. Immunol. 100, 277-283). TNF-α was selected as it is one of the most potent and well studied activators of NF-κB (May and Ghosh, 1997,Sem. Cancer Biol. 8, 63-73). IFN-γ exerts its effects on gene regulation through proteins which bind the ISREs (Johnson and Pober, 1994, Mol. Cell. Biol. 14, 1322-1332). The intracellular signalling mechanisms of IL-4 are distinct from those of TNF and the ability of this cytokine to induce DPP-IV may be explained by an interaction between the NF-κB and STAT6 transcription factors (Ichiki

et al., 1993,. Immunol. 150, 5408-5417; Messner et al., 1997, J. Immunol. 159, 3330-3337).

Materials and Methods:

Human melanoma cells MEL-22a were treated for 72 hours with the following recombinant cytokines; IL-4 (specific activity $1X10^5$ U/mg), IFN-γ ($2X10^7$ U/MG) and TNF-α ($9X10^6$ U/mg). Total cell lysates were extracted using lysis buffer and were assayed for dipeptidyl peptidase enzyme activities. (For details regarding extraction of cell lysates and enzyme assay, see materials and methods in the previous section).

The results of the experiments show that IL-4, TNF-α and IFN-γ can each increase functional DPP-IV activity by at least two fold in cultured melanoma cells. The induction of DPP-IV was accompanied by significant growth inhibition and eventual apoptosis, demonstrating a reversal of the malignant phenotype.

WHAT IS CLAIMED IS:

1. A method of suppressing the malignant phenotype of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein, thereby suppressing the malignant phenotype of the cancer.

2. A method of suppressing the malignant phenotype of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent, thereby suppressing the malignant phenotype of the cancer in the subject.

3. A method of inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein, thereby inducing apoptosis.

4. A method of inducing apoptosis of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a dipeptidyl peptidase IV protein, a nucleic acid encoding a fragment of a dipeptidyl peptidase IV protein, or the nucleic acid encoding a mutant dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent, thereby inducing apoptosis.

5. A method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective

amount of a purified dipeptidyl peptidase IV protein, a purified fragment of a dipeptidyl peptidase IV protein, a purified mutant dipeptidyl peptidase IV, or a purified chimeric dipeptidyl peptidase IV protein and a pharmaceutical acceptable carrier or diluent.

6. The method of claim 5, wherein the chimeric is a fusion protein.

7. The method of claim 5, wherein the fusion protein comprises dipeptidyl peptidase IV, a fragment of dipeptidyl peptidase IV, or a mutant dipeptidyl peptidase IV; and a Fc region of immunoglobulin, or a fragment thereof.

8. The method of claim 5, wherein the fusion protein comprises dipeptidyl peptidase IV, a fragment of dipeptidyl peptidase IV, or a mutant dipeptidyl peptidase IV linked to a antibody, or fragment thereof.

9. A method of inducing expression of dipeptidyl peptidase IV in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

10. The method of claim 9, wherein the agent regulates the activation of a response element of the dipeptidyl peptidase IV gene.

11. The method of claim 10, wherein the response element is: interferon stimulated response elements (ISREs), butyrate response elements, NF-kB, c-myc, or c-myb.

12. The method of claim 9, wherein the agent is a cytokine or growth factor.

13. The method of claim 10, wherein the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ.

14. A method of treating a subject with cancer which comprises, administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the dipeptidyl peptidase IV gene and a pharmaceutical acceptable carrier or diluent.

15. The method of claim 14, wherein the agent regulates the activation of a response element of the dipeptidyl peptidase IV gene.

16. The method of claim 15, wherein the response element is: interferon stimulated response elements (ISREs), butyrate response elements, NF-kB, c-myc, or c-myb.

17. The method of claim 14, wherein the agent is a cytokine or growth factor.

18. The method of claim 17, wherein the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ.

19. The method of claim 1, wherein the cancer is selected from a group consisting of: melanoma; lymphoma; leukemia; and prostate, colorectal, pancreatic, breast, brain, or gastric carcinoma.

20. The method of claim 19, wherein the pharmaceutical composition is administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermaly, subcutaneously, intraperitonealy, intraventricularly, or intracranialy.

21. The method of claim 20, wherein the pharmaceutical composition is delivered as naked DNA, within a viral vector, or within a liposome

22. The method of claim 21, wherein the viral vector is a retrovirus, adeno-associated virus, or adenovirus.

23. A method of suppressing the malignant phenotype of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α , thereby suppressing the malignant phenotype of the cancer.

24. A method of suppressing the malignant phenotype of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent, thereby suppressing the malignant phenotype of the cancer in the subject.

25. A method of inducing apoptosis of cancer cells in a subject, comprising introducing into the cancer cell an amount of a nucleic acid encoding a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α, thereby inducing apoptosis.

26. A method of inducing apoptosis of cancer cells in a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a fibroblast activating protein-α (FAP-α), a nucleic acid encoding a fragment of a fibroblast activating protein-α, or the nucleic acid encoding a mutant fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent, thereby inducing apoptosis.

27. A method of treating a subject with cancer which comprises administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a purified fibroblast activating protein-α , a purified fragment of a fibroblast activating protein-α, a purified mutant fibroblast activating protein-α,

or a purified chimeric fibroblast activating protein-α and a pharmaceutical acceptable carrier or diluent.

28. The method of claim 27, wherein the chimeric is a fusion protein.

29. The method of claim 27, wherein the fusion protein comprises fibroblast activating protein, a fragment of fibroblast activating protein-α,or a mutant fibroblast activating protein-α; and a Fc region of immunoglobulin, or a fragment thereof.

30. The method of claim 27, wherein the fusion protein comprises fibroblast activating protein-α a fragment of fibroblast activating protein-α, or a mutant fibroblast activating protein-α linked to a antibody, or fragment thereof.

31. A method of inducing expression of fibroblast activating protein-α in cancer cells of a subject, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the fibroblast activating protein-α gene and a pharmaceutical acceptable carrier or diluent.

32. The method of claim 31, wherein the agent regulates the activation of a response element of the fibroblast activating protein-α gene.

33. The method of claim 31, wherein the agent is a cytokine or growth factor.

34. The method of claim 33, wherein the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ.

35. A method of treating a subject with cancer which comprises, administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of an agent capable of activating transcription of the fibroblast activating protein-α gene and a pharmaceutical acceptable carrier or diluent.

36. The method of claim 35, wherein the agent regulates the activation of a response element of the fibroblast activating protein-α gene.

37. The method of claim 36, wherein the agent is a cytokine or growth factor.

38. The method of claim 37, wherein the cytokine or growth factor is: interleukin-4, tumor necrosis factor-α, or interferon-γ.

39. The method of claim 23, wherein the cancer cells are selected from a group consisting of: melanoma; lymphoma; leukemia; and prostate, colorectal, pancreatic, breast, brain, or gastric carcinoma.

40. The method of claim 39, wherein the pharmaceutical composition is administered parenterally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermaly, subcutaneously, intraperitonealy, intraventricularly, or intracranialy.

41. The method of claim 40, wherein the pharmaceutical composition is delivered as naked DNA, within a viral vector, or within a liposome.

42. The method of claim 41, wherein the viral vector is a retrovirus,adeno-associated virus, or adenovirus.

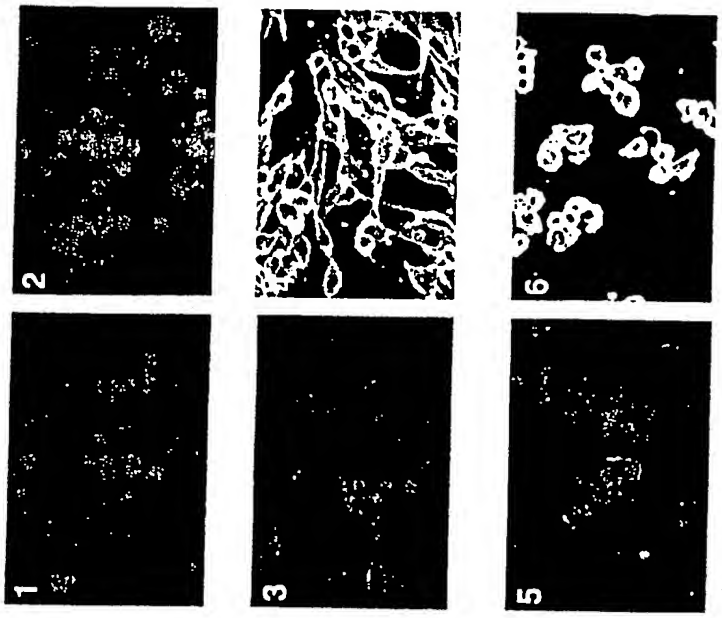


Figure 1A

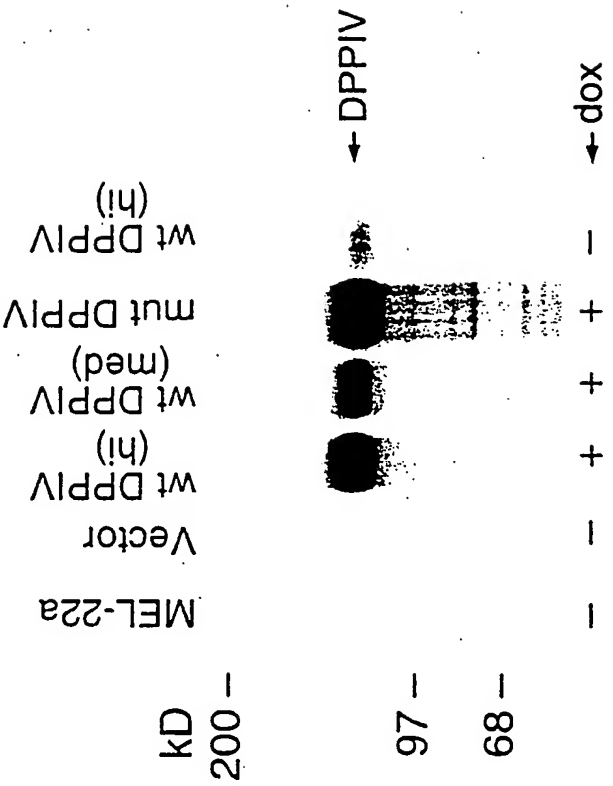

kD
200 –
97 –
68 –
MEL-22a
Vector
wt DPPIV (hi)
wt DPPIV (med)
mut DPPIV
wt DPPIV (hi)
- DPPIV
- dox
- - + + + -

Figure 1B

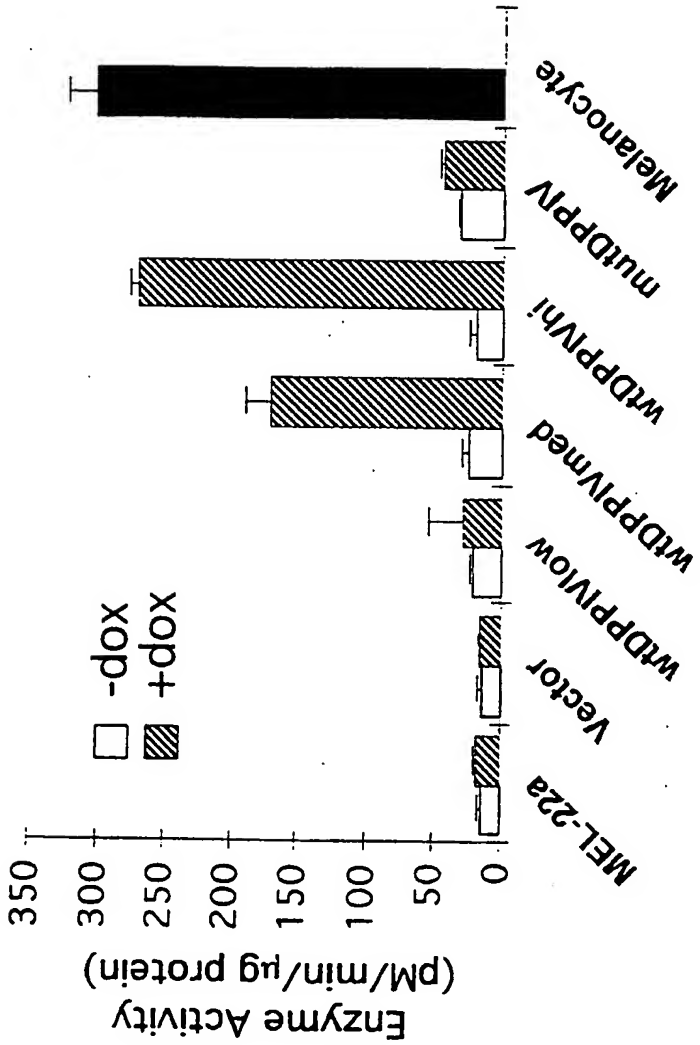

Enzyme Activity
(pM/min/µg protein)
350
300
250
200
150
100
50
0
-dox
+dox
MEL-22a
Vector
wtDPPIVlow
wtDPPIVmed
wtDPPIVhi
mutDPPIV
Melanocyte

Figure 1C

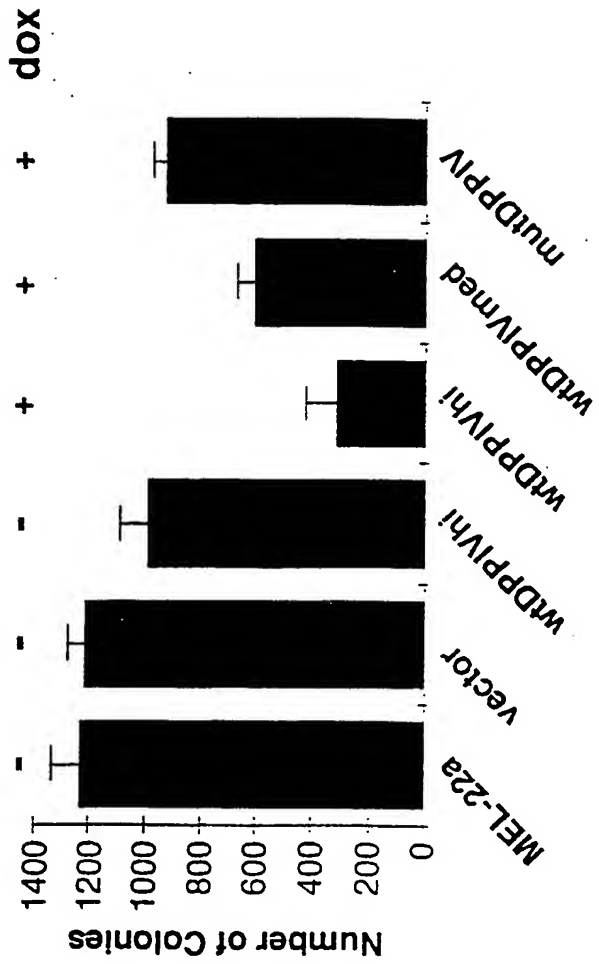
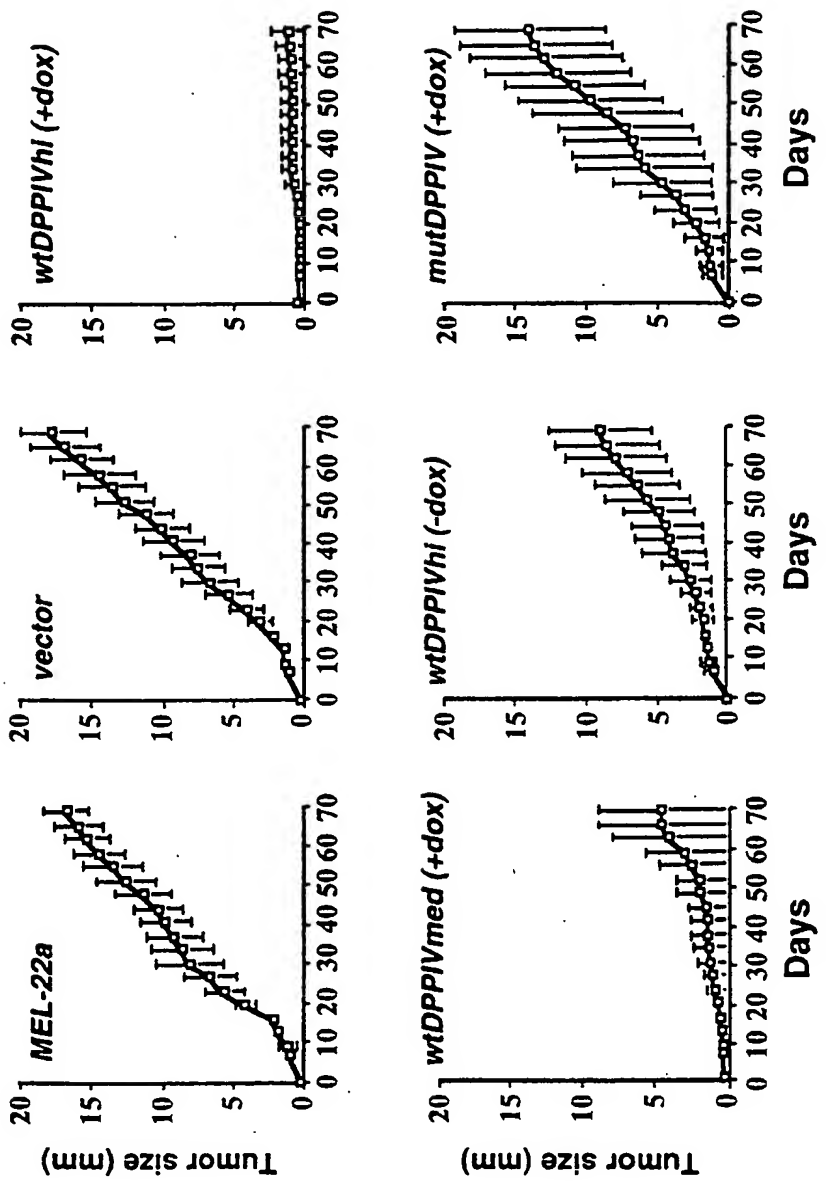

dox
- - - + + +
MEL-22a
vector
wtDPPIVhi
wtDPPIVhi
wtDPPIVmed
mutDPPIV
0 200 400 600 800 1000 1200 1400
Number of Colonies

Figure 2


MEL-22a
vector
wtDPPIVhi (+dox)
wtDPPIVmed (+dox)
wtDPPIVhi (-dox)
mutDPPIV (+dox)
Tumor size (mm)
Days
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0 5 10 15 20

Figure 3A

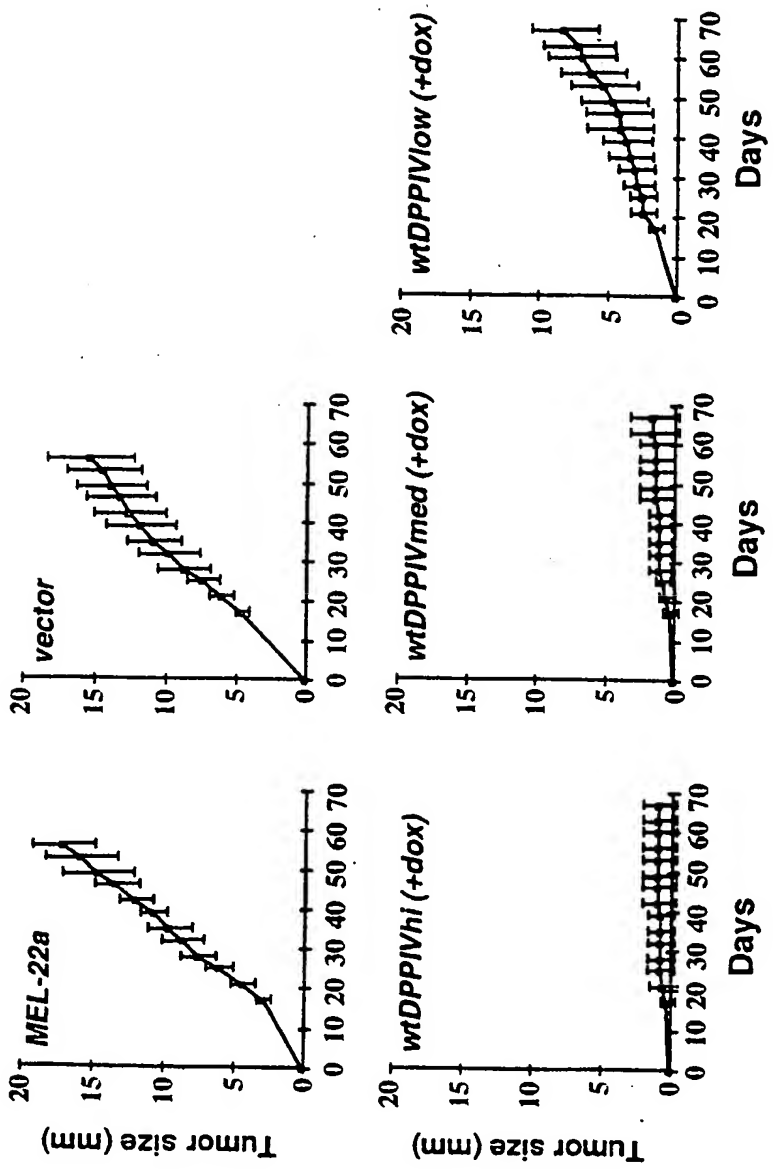

MEL-22a
vector
wtDPPIVhi (+dox)
wtDPPIVmed (+dox)
wtDPPIVlow (+dox)
Tumor size (mm)
Days
0
5
10
15
20
0 10 20 30 40 50 60 70

Figure 3B

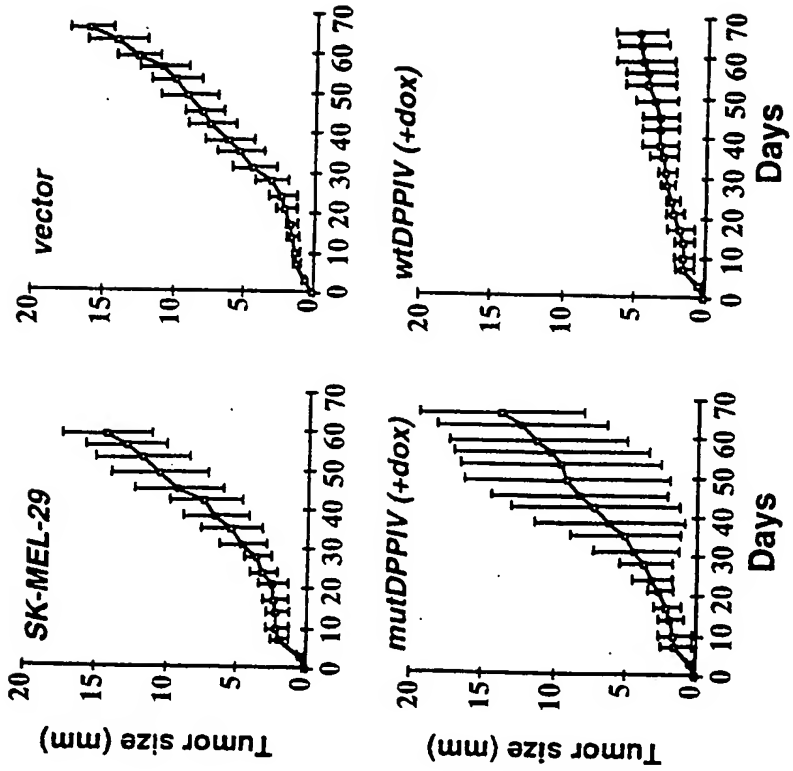

SK-MEL-29
vector
mutDPPIV (+dox)
wtDPPIV (+dox)
Tumor size (mm)
Days
0
5
10
15
20
0 10 20 30 40 50 60 70

Figure 3C

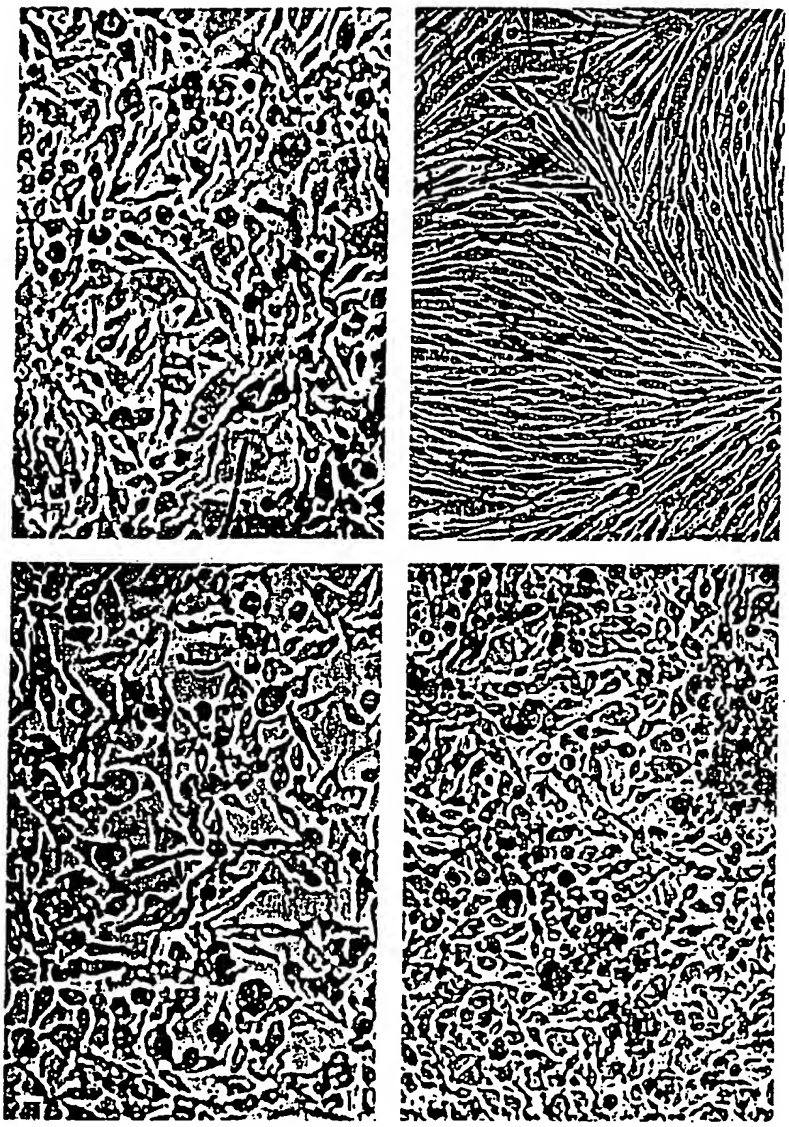
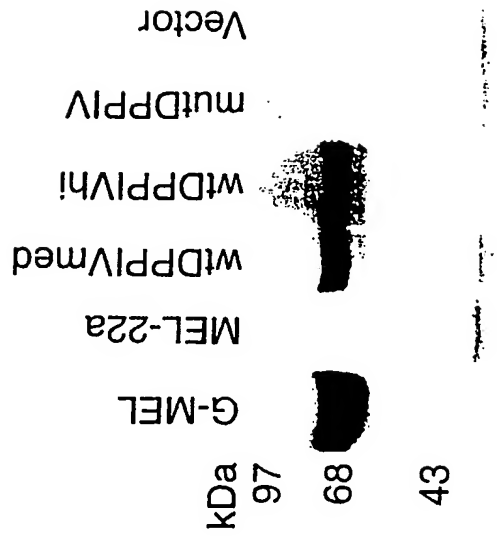


Figure 4A

Figure 4B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68
43
G-MEL
MEL-22a
wtDPPIVmed
wtDPPIVhi
mutDPPIV
Vector

Figure 4C

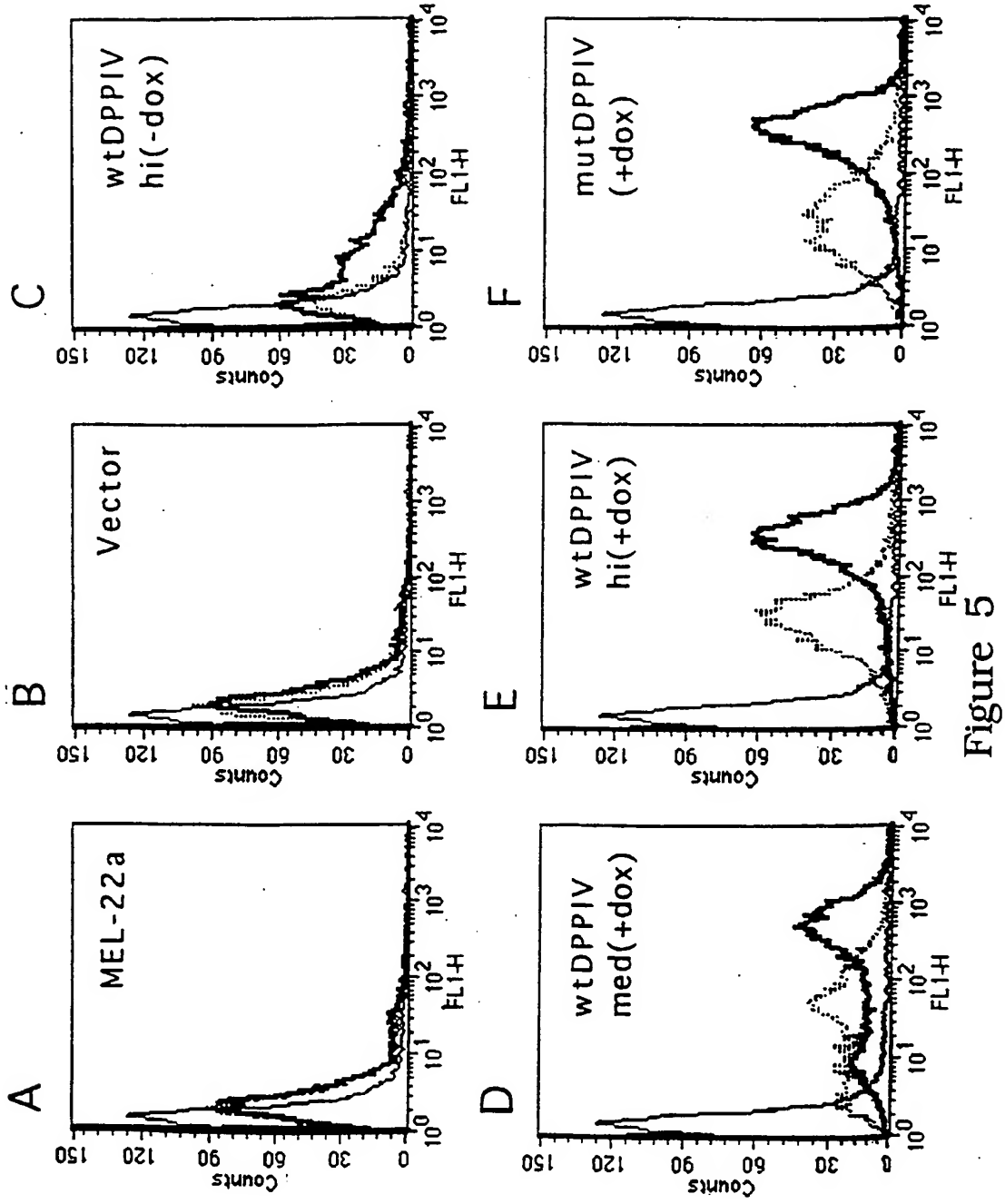
A
MEL-22a
B
Vector
C
wtDPPIV
hi(-dox)
D
wtDPPIV
med(+dox)
E
wtDPPIV
hi(+dox)
F
mutDPPIV
(+dox)
Counts
FL1-H

Figure 5